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Short Communication

Absence of Fluorescence Quenching in a Photosynthetic Mutant of Euglena gracilis[1]

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Substantial experimental evidence indicates that the level of chlorophyll fluorescence in algae and higher plants is controlled predominantly by the activity of photosystem II (PS II) of the photosynthetic electron transport chain (2). The overall fluorescence yield seems to depend on the oxidation/reduction state of Q, the presumed primary electron acceptor of PS II (7) ($H_2O \rightarrow ? \rightarrow Y \xrightarrow[\text{PS II}]{h\nu} Q \rightarrow A \rightarrow ? \rightarrow P_{700} \xrightarrow[\text{PS I}]{h\nu} XH$). According to Duysens' hypothesis (4), the oxidized form of Q quenches chlorophyll fluorescence, while the reduced form (QH) does not. We report here a photosynthetically deficient strain of *Euglena* which appears to be lacking the fluorescence quenching normally associated with the presence of Q.

Euglena gracilis (strain Z) was cultured as previously described (9). The pale green mutant P_4 is unable to carry out photosynthetic electron transport because of a block at or near light reaction II; detailed studies of this strain have been reported elsewhere (10).

Chloroplast fragments were prepared according to Katoh and San Pietro (6). Fluorescence was measured with the technique devised by Duysens (3). Whole cells or chloroplasts were illuminated with a modulated monochromatic light beam (λ = 435 nm, 270 cps), and the fluorescence emission was measured with a photomultiplier/amplifier system tuned to the chopping frequency. Provision was also made for illuminating the sample with a constant actinic beam (510 ± 40 nm; I = 5×10^3 ergs cm^{-2} sec^{-1}); fluorescence excited by this light was not detected. With this apparatus the relative fluorescence yield could be measured in the presence and absence of actinic light.

Fig. 1 illustrates the relationship between fluorescence and chlorophyll concentration for whole cells of *Euglena*. The fluorescence of P_4 was consistently 3 to 5 times higher than the wild type. We attribute this to the absence of fluorescence quenching in whole cells of the mutant strain. Wild type fluorescence could be increased 2 to 3-fold by the addition of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethyl urea] to a final concentration of 10 μM. Fluorescence emission spectra of wild type and P_4 at room temperature showed maxima at 685 nm.

The effect of actinic illumination on the relative fluorescence yield was studied in isolated chloroplast fragments. Illumination of wild type fragments with a very weak measuring beam (20 ergs cm^{-2} sec^{-1})

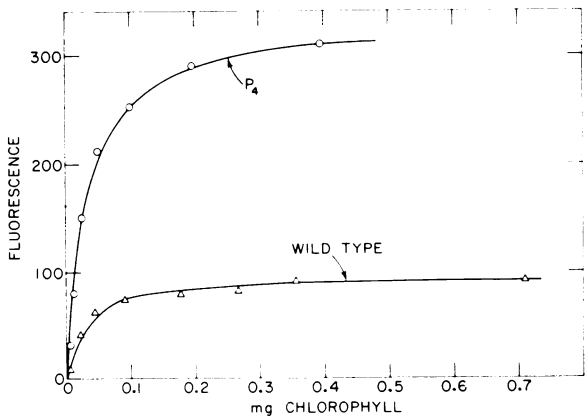

FIG. 1. Fluorescence of whole cells of *Euglena* at 685 nm *versus* chlorophyll concentration (mg/ ml). The modulated light intensity was 10^3 ergs cm^{-2} sec^{-1}.

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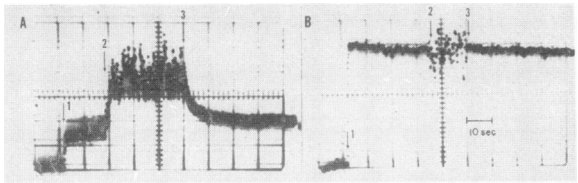

FIG. 2. The effect of actinic illumination on the relative fluorescence yield of wild type and P_1 chloroplasts. The modulated light intensity was 20 ergs cm^{-2} sec^{-1}. The intensity of the actinic beam was 5×10^3 ergs cm^{-2} sec^{-1}. The measuring beam was turned on at the arrow marked 1 above. The actinic beam was turned on at 2 and off at 3. A) Wild type chloroplasts. B) P_1 chloroplasts.

excited a low level of fluorescence (Fig. 2a). Subsequent illumination of the sample with actinic light increased the fluorescence yield by a factor of 2.5, presumably by bringing about higher steady state reduction of Q (Fig. 2a). Comparable experiments with P_1 chloroplast fragments clearly demonstrated the absence of fluorescence quenching in the mutant strain. The low intensity measuring beam induced a higher level of fluorescence than that seen in wild type, and the actinic light had no effect on the fluorescence yield (Fig. 2b).

The kinetics of fluorescence emission by isolated plastid fragments are shown in Fig. 3. The biphasic curve shown for wild type plastids (Fig. 3a) is typical of many photosynthetic systems (7). The initial rise (F_o) represents constant chlorophyll fluorescence which is unaffected by Q. The second (or variable) phase is related to the state of oxidation of Q. The time course shown in Fig. 3a demonstrates the attainment of a steady state in which Q is partially reduced. Addition of DCMU to a final concentration of 10 μM altered the kinetics of induction and increased the final yield (Fig. 3a). Since DCMU is believed to block electron transport between Q and A, the secondary electron acceptor (2), the increase of fluorescence with time in Fig. 3b represents the kinetics of Q reduction without subsequent reoxidation of QH be photosystem I. The final fluorescence yield reflects almost complete absence of fluorescence quenching.

No initial rate of increase in fluorescence could be obtained with P_1 plastids even in very weak measuring light (Fig. 3c); maximum fluorescence was attained within the time required for shutter opening on the measuring beam (*ca.* 25 msec). In DCMU-treated wild type, 4 to 5 sec were required for attainment of maximum fluorescence (Fig. 3b). DCMU had no effect on either the fluorescence yield or the rate of attainment of the final yield in P_1 (Fig. 3c).

Within the context of the Duysens hypothesis, we conclude that 1) Q is missing altogether in P_1, *or* 2) Q is permanently reduced in P_1, *or* 3) the rate of Q reduction is many times higher in P_1, and the kinetics of reduction can not be detected under the conditions of our experiments, *or* 4) Q is present in P_1 but unable to quench chlorophyll fluorescence for various reasons (*e.g.* mutational alteration of chloroplast structure in P_1). Explanation 2 is rendered very improbable by the existence of pathways of QH reoxidation in the dark even in the presence of DCMU (1). We have no evidence either to support or reject explanation 4. We favor the interpretation that the mutant strain may be missing Q, the primary oxidant of PS II of photosynthesis.

Several investigators have reported increased fluorescence yields in photosynthetic mutants of a variety of organisms. Mutant IIδ of *Oenothera* recently described by Fork and Heber (5) shows constant high fluorescence, and many of the properties of this mutant are similar to P_1. The fluorescence of mutant 11 of *Scenedesmus* is unaffected by

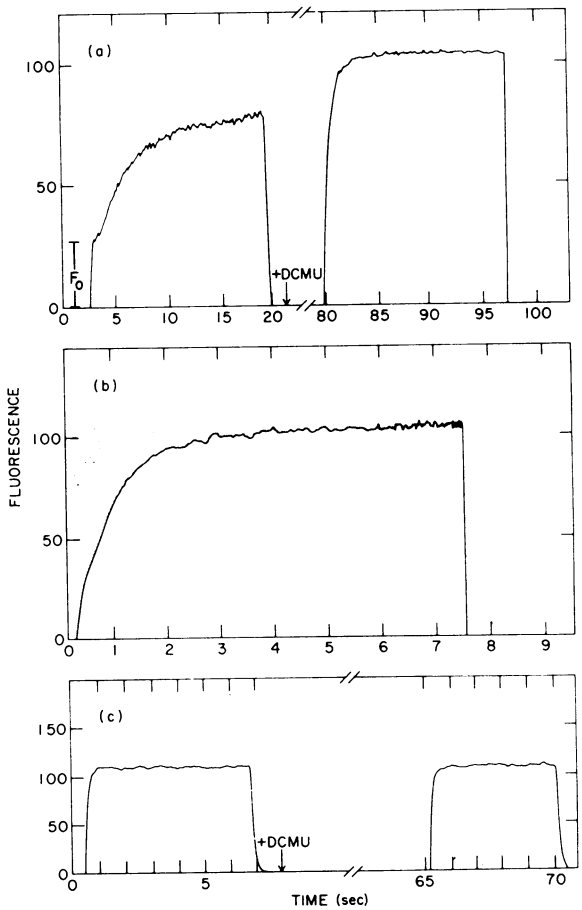

FIG. 3. Fluorescence kinetics of *Euglena* chloroplasts at 685 nm. The modulated light intensity was 260 ergs cm^{-2} sec^{-1}. a) Wild type chloroplasts. The initial fluorescence (F_o) is indicated on the graph. DCMU was added at the indicated time to give a final concentration of 10 μM. b) Wild type chloroplasts in the presence of 10 μM DCMU. The time scale is expanded to illustrate the kinetics of fluorescence emission. c) P_1 chloroplasts. DCMU was added at the indicated time to give a final concentration of 10 μM.

actinic illumination (1) but the fluorescence kinetics have not been reported. Butler (2) has suggested that this mutant may be missing Q. Recently Lavorel and Levine (8) have described the characteristics of a mutant strain of *Chlamydomonas reinhardi* (*ac-115*) which appears to be lacking Q, but which also lacks detectable amounts of cytochrome 559; further investigations will be necessary to determine the exact nature of the lesion(s) in the mutant. The finding of mutant organisms apparently lacking Q opens the possibility of identifying the chemical nature of this substance.

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